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54. (New) A method of preparing a composition comprising leukemia inhibitory factor (LIF) or a derivative or homologue thereof which exhibits improved chemical or physical stability of LIF, said method comprising admixing LIF or its derivative or homologue with a stabilizing agent.

55. (New) A method according to claim 42 wherein the pH is between about 4.5 and about 6.0.

REMARKS

In the Official Action dated May 9, 2001, Claims 1-43 have been rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite. Claims 1-3, 6-13, 15, 16, 20-29 and 32-40 have been rejected under 35 U.S.C. §102(e) as allegedly anticipated by Patterson, et al., U.S. Patent No. 6,156,729 (hereinafter "Patterson, et al.") as evidenced by Cleland, et al. (1993) Crit. Rev. Therapeutic Drug Carrier Systems, 10:307-377 (hereinafter "Cleland, et al."). Claims 4, 5, 14, 17-19, 30, 31 and 43 have been rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Patterson, et al. in view of Cleland, et al.

This Response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claims 1-43 have been rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite. Claims 1, 6, 21 and 33 have been rejected as allegedly indefinite because the Claims are drawn to "derivatives or homologs of LIF". Applicants respectfully direct the Examiner's attention to the specification of Page 4, Line 19 – Page 5, Line 7 which provides a specific description of derivatives of LIF which are contemplated by the specification and

no page 4

readily understood by the skilled artisan. Moreover, the present specification contemplates that derivatives and homologues of LIF will maintain the characteristic functionality of native LIF.

Claim 6 has been rejected as allegedly drawn to compositions comprising "functional equivalents of a surfactant". In an effort to expedite favorable prosecution, the term surfactant has been removed from Claim 6.

Claim 7 has been rejected as allegedly indefinite as drawn to a composition comprising a "buffer species". The Examiner alleges that the claim is unclear because the specification does not provide a closed definition of what compounds are encompassed by the term "buffer species." Applicants respectfully direct the Examiner's attention to the specification at Page 11, lines 18-20 wherein suitable buffers (i.e., phosphate, citrate and acetate) are specifically described. Claim 7 has also been rejected as allegedly indefinite because it is drawn to a composition comprising a "polymeric compound." Applicants specifically direct the Examiner's attention to the specification at Page 11, lines 12-16 wherein several polymeric compounds are specifically provided.

Claims 11, 27 and 38 have been rejected as allegedly drawn to compositions or methods comprising a "polyoxyethylene derivative". The Examiner specifically alleges that it is "unclear what chemical compounds are encompassed by a polyoxyethylene derivative". Applicants respectfully submit that polyoxyethylene derivatives are well known and commonly found in e.g., The Handbook of Pharmaceutical Excipients, 2nd Edition, Eds., A. Wade and P.J. Weller, Pharmaceutical Press, London, 1994. Applicants respectfully submit that The Handbook of Pharmaceutical Excipients was and is available to pharmaceutical

formulators and is commonly used to locate information about pharmaceutically acceptable excipients and/or additives such as polyoxyethylene derivatives.

Claims 33-42 have been rejected as allegedly lacking steps involved in the method or process. The Examiner has also rejected Claims 33-42 under 35 U.S.C. §101 as allegedly reciting a use, without setting forth any steps in the process. Applicants have cancelled Claim 33 and added Claim 54. Claims 54 and 34-42 are in accord with U.S. Patent Law and recite a method of preparing a stable LIF composition, which sets forth the positive steps delimiting how the process is practiced.

Accordingly, the rejection of Claims 1-43 under 35 U.S.C. §112, second paragraph is overcome and withdrawal thereof is respectfully requested.

Claims 1-3, 6-13, 15, 16, 20-29 and 32-40 have been rejected under 35 U.S.C. §102(e) as allegedly anticipated by Patterson as evidenced by Cleland. Claims 4, 5, 14, 17-19, 31 and 43 have been rejected as allegedly unpatentable under 35 U.S.C. §103(a) over Patterson et al. in view of Cleland. In response to the rejections, Applicants proffer the Declaration of Dr. Susan Ann Charman pursuant to 37 C.F.R. §1.131 which obviates the Patterson, et al. reference and thus the rejection under 35 U.S.C. §102(e) and 103(a). Applicants respectfully submit that the §1.131 Declaration evidences the reduction to practice of the claimed invention prior to the availability of teachings of the cited reference to Patterson, et al. Thus, the rejection of Claims 1-3, 6-13, 15, 16, 20-29 and 32-40 under 35 U.S.C. §102(e) and the rejection of Claims 4, 5, 14, 17-19, 31 and 43 under 35 U.S.C. §103(a) are overcome and withdrawal thereof is respectfully requested.

Even assuming pro arguendo the applicability of Patterson et al. as a proper

reference, the teachings of Cleland do not suggest the present invention. Cleland provides a general review of the development of stable protein formulations. Cleland does not even mention compositions comprising LIF having a pH between about 3.5 and 6.5. Applicants respectfully submit that Cleland completely fails to motivate the skilled artisan to prepare a composition or practice the methods according to the present invention. Accordingly, a prima facie case of obviousness has not been established.

New Claims 44-55 have been added to further define the subject matter to which applicants are entitled. The support for added Claims 44 and 45 is found throughout the specification and in original Claims 4 and 5. Support for Claim 46 is found throughout the specification and in original Claim 6 as filed. Support for Claim 47 is found throughout the specification and particularly at Page 11, lines 13-14, for example. Support for Claims 48 and 49 is found throughout the specification and particularly Page 10, lines 21-25, for example. Support for Claim 50 is found throughout the specification and particularly at Page 11, lines 12-14, for example. Support for Claim 51 is found throughout the specification and particularly at Page 11, line 13, for example. Support for Claims 52 and 53 is found throughout the specification and particularly at Page 4, line 5, and Page 14, lines 11-12, for example. Support for Claim 54 is found in Claim 33 as originally filed and support for Claim 55 is found throughout the specification and particularly at Page 4, line 5, and Page 14, lines 11-12, for example.

In addition, applicants have corrected inadvertent typographical errors throughout the specification and in this regard provide a clean copy (Exhibit 1) and a marked-up version (Exhibit 2), of the corrections to facilitate modifications to the specification by the

Office. In addition, Tables 20 and 21 have been amended to clarify what appears to be confusing information owing to the use of two different ion exchange columns in Tables 20 and 21, as originally filed. Applicants have therefore converted the data therein to percentages of the initial concentrations remaining to facilitate a comparison between the different formulations. Accordingly, the results shown in Tables 20 and 21 are expressed as a percentage of the initial concentration remaining after the storage period indicated. Support for the amendments to Tables 20 and 21 is found throughout the specification and particularly at Page 49, lines 22-27. No new matter has been added.

As indicated, attached is a marked-up version of the changes made to the specification and claims (Exhibit 2) by the current amendment. The marked-up version of the changes made to the specification is captioned "Version showing were changes have occurred". The attached page providing changes made to the claims is captioned "Version with markings to show changes made".

Thus, in view of the foregoing amendments and remarks, applicants respectfully submit that the present application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Please cancel Claims 4, 5, 19, 27, 31 and 33 without prejudice.

Please amend the following claims as follows:

1. (Amended) A composition comprising leukaemia inhibitory factor (LIF) or a derivative or homologue thereof and a stabilizing agent [facilitating chemical and/or physical stability of LIF in the composition], additives for maintaining pH and isotonicity and one or more pharmaceutically acceptable carriers [and/or diluents] wherein the pH of the composition is between about 3.5 and 6.5.

2. (Amended) A composition according to [claim] any one of claims 1 or 44 or 45 wherein the stabilizing agent facilitates reduced aggregation of LIF.

3. (Amended) A composition according to [claim] any one of claims 1 or 44 or 45 wherein the stabilizing agent facilitates a reduction in the deamidation of LIF.

6. (Amended) A composition according to claim 1 [or 5] wherein the stabilizing agent is an [isotonicity agent, an] agent which increases or maintains the conformational stability of LIF or its derivatives of homologues or [a surfactant or] functional equivalents thereof.

7. (Amended) A composition according to claim 6 wherein the stabilizing agent is [an isotonicity agent] selected from a polyhydric alcohol, a pharmaceutically acceptable salt, a buffer species, a sugar and a pharmaceutically acceptable polymeric compound.

14. (Amended) A composition comprising leukaemia inhibitory factor (LIF), additives for maintaining pH and isotonicity and one or more pharmaceutically acceptable carriers and/or diluents and wherein the composition has a pH of between about 3.5 and 6.5.

15. (Amended) A composition according to claim [6] 14 wherein the aggregation of LIF [over time] is reduced over time.

16. (Amended) A composition according to claim [6 or 7] 14 or 48 or 49 wherein the deamidation of LIF [over time] is reduced over time.

17. (Amended) A composition according to claim 14 or 48 or 49 where the pH is maintained by the presence of a buffer species selection from a phosphate, citrate and acetate buffer.

21. (Amended) A method for preparing a composition comprising leukaemia inhibitory factor (LIF) or a derivative or homologue thereof and which exhibits reduced deamidation and/or [agglutination] aggregation of LIF or its derivative or homologues over time said method comprising admixing LIF or its derivative or homologue with a stabilizing agent and additives for maintaining pH and isotonicity.

22. (Amended) A method according to claim 21 wherein the stabilizing agent is [a isotonicity agent,] an agent which increases or maintains the conformational stability of LIF or its derivatives or homologues or a surfactant or functional equivalents thereof.

23. (Amended) A method according to claim 22 wherein the stabilizing agent is [an isotonicity agent] selected from a polyhydric alcohol, a pharmaceutically acceptable salt, a buffer species, a sugar and a pharmaceutically acceptable polymeric compound.

28. (Amended) A method according to claim [23] 21 wherein the [buffer species] additives for maintaining pH and isotonicty are [is] selected from a phosphate, citrate and acetate buffer.

29. (Amended) A method according to claim 28 wherein the [buffer species is] additives for maintaining pH and isotonicity are [a] citrate or acetate buffer.

30. (Amended) A method according to [any of claims 22 to 29] claim 21 further comprising adjusting the pH to between [from] about 3.5 and about 6.5.

32. (Amended) A method according to [any one of the claims 22 to 31] claim 21 further comprising admixing one or more pharmaceutically acceptable carriers and/or diluents.

34. (Amended) [Use] A method according to claim [33] 54 wherein the stabiling agent is [an isotonicity agent] selected from a polyhydric alcohol, a pharmaceutically acceptable salt, a buffer species, a sugar and a pharmaceutically acceptable polymeric compound.

35. (Amended) [Use] A method according to claim 34 wherein the polyhydric alcohol is sorbitol.

36. (Amended) [Use] A method according to claim 34 wherein the surfactant is an anionic, cationic, amphoteric or non-ionic surfacant.

37. (Amended) [Use] A method according to claim 36 wherein the surfactant is selected from a fatty alcohol, glyceryl ester and a fatty acid ester of a fatty alcohol or other alcohol.

38. (Amended) [Use] A method according to claim [33] 54 where the stabilizing agent is selected from a polysorbate, a polyoxyethylene derivative or a pharmaceutically acceptable polyoxyethylene-polyoxypropylene copolymer.

39. (Amended) [Use] A method according to claim 34 wherein the buffer species is selected from a phosphate, citrate and acetate buffer.

40. (Amended) [Use] A method according to claim 39 wherein the buffer species [isa] is a citrate or acetate buffer.

41. (Amended) [Use] A method according to [any one of claims 33 to 40] claim 54 where the pH of the composition is between [from] about 3.5 to about 6.5.

42. (Amended) [Use] A method according to [any one of claims] claim 41 wherein the pH is between [from] about 4.5 and about 5.5

43. (Amended) A composition according to claim [14] 13 wherein LIF is present in an amount from about 0.1 μg/ml to about 100 mg/ml.

Please add the following new Claims 44-55:

44. (New) A composition according to claim 1 wherein the pH of the composition is between about 4.5 and 6.5.

45. (New) A composition according to claim 2 wherein the pH of the composition is between about 4.5 and 6.0.

46. (New) A composition according to claim 6 wherein the stabilizing agent is a surfactant.

47. (New) A composition according to claim 9 wherein the stabilizing agent is polysorbate 20 and/or polysorbate 80.

48. (New) A composition according to claim 14 wherein the composition has a pH of between about 4.5 and 6.5.

49. (New) A composition according to claim 48 wherein the composition has a pH of between about 4.5 and 6.0.

50. (New) A method according to claim 23 wherein the stabilizing agent is selected from a polysorbate, a polyoxyethylene derivative and a pharmaceutically acceptable polyoxyethylene-polyoxypropylene copolymer.

51. (New) A method according to claim 50 wherein the polysorbate is polysorbate 20 and/or polysorbate 80.

52. (New) A method according to claim 30 further comprising adjusting the pH to between about 4.5 and about 6.5.

53. (New) A method according to claim 30 further comprising adjusting the pH to between about 4.5 and 6.0.

54. (New) A method of preparing a composition comprising leukemia inhibitory factor (LIF) or a derivative or homologue thereof which exhibits improved chemical or physical stability of LIF, said method comprising admixing LIF or its derivative or homologue with a stabilizing agent.

55. (New) A method according to claim 42 wherein the pH is between about 4.5 and about 6.0.

Version showing where changes have occurred

COMPOSITIONS OF LEUKAEMIA INHIBITORY FACTOR

FIELD OF THE INVENTION

The present invention relates generally to compositions and more particularly to compositions comprising leukaemia inhibitory factor (hereinafter referred to as "LIF") or derivative or homologues thereof. The compositions of the present invention are particularly useful as compositions which exhibit enhanced stability and/or which exhibit reduced aggregation and/or reduced deamidation of LIF, its derivatives or other active ingredients.

BACKGROUND OF THE INVENTION

LIF is a polyfunctional glycoprotein with diverse actions on a broad range of tissue and cell types, including induction of differentiation in a number of myeloid leukaemic cell lines, suppression of differentiation in normal embryonic stem cells, stimulation of proliferation of osteoblasts and DA-1 haemopoietic cells and potentiation of the of the proliferative action of interleukin-3 (IL-3) on megakaryocyte precursors. Functionally, LIF is able to switch autonomic nerve signalling from adrenergic to cholinergic mode, stimulate calcium release from bones, stimulate the production of acute phase proteins by hepatocytes and induce loss of fat deposits by inhibiting lipoprotein lipase-mediated lipid transport into adipocytes.

With a potentially broad range of clinical applications, it is imperative that compositions containing LIF are presented in a stable form and remain so during an extended period which may include shipment, handling and storage. Thus, a stable composition is one which retains its physical, chemical, therapeutic and toxicological profile over this period.

Deamidation is the most significant chemical degradation of LIF over time. It is clearly desirable that this process is minimized. Adsorption of LIF onto surfaces of containers, vials, syringes and infusion tubing is also a potential problem and must be minimized to ensure accurate dose and concentration. Physical degradation, such as aggregation or flocculation, may occur due to denaturation caused by elevated temperatures and/or

agitation and excessive handling of the composition. Such degradation is clearly undesirable in terms of appearance and more importantly, consistent and effective administration of LIF in clinical applications. Storage at temperatures below room temperature typically retards chemical degradation, with storage in the frozen state being generally the most effective. Whilst this may minimize chemical degradation, the process of thawing the composition may then result in aggregation.

Thus, there exists a need for a stable composition and, in particular, a stable pharmaceutical composition of LIF and/or its derivatives or homologues wherein chemical and physical degradation is minimised.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

One aspect of the present invention contemplates a composition comprising leukaemia inhibitory factor (LIF) or a derivative or homologue thereof and a stabilizing agent facilitating chemical and/or physical stability of LIF in the composition, additives for maintaining pH and isotonicity and one or more pharmaceutically acceptable carriers and/or diluents.

Another aspect of the invention provides a composition with improved chemical and physical stability comprising LIF or a derivative or homologue thereof, a stabilizing agent, additives for maintaining pH and isotonicity and one or more pharmaceutically acceptable carriers or diluents under conditions in which aggregation of LIF is reduced.

Yet another aspect of the invention provides a composition with improved chemical and physical stability comprising LIF or a derivative or homologue thereof, a stabilizing agent, additives for maintaining pH and isotonicity and one or more pharmaceutically acceptable carriers or diluents under conditions in which deamidation of LIF is reduced.

Still another aspect the present invention is directed to a stable composition comprising LIF or a derivative or homologue thereof, together with one or more pharmaceutically acceptable carriers or diluents, wherein the composition has a pH of between about 3.5 and about 6.5.

A further aspect the present invention provides a stable composition comprising LIF or a derivative or homologue thereof, together with one or more pharmaceutically acceptable carriers or diluents, wherein the composition has a pH of between about 3.5 and about 6.5 under conditions in which aggregation of LIF is reduced.

Another aspect the present invention contemplates a stable composition comprising LIF or a derivative or homologue thereof, together with one or more pharmaceutically acceptable carriers or diluents, wherein the composition has a pH of between about 3.5 and about 6.5 under conditions in which deamidation of LIF is reduced.

Yet another aspect of the present invention contemplates a method for preparing a composition comprising Leukaemia Inhibition Factor (LIF) or a derivative or homologue thereof and which exhibits reduced deamidation and/or ~~agglutination or~~aggregation of LIF or a derivative or homologue over time said method comprising admixing LIF or its derivative or homologue with a stabilizing agent.

Still another aspect of the present invention is directed to the use of a stabilizing agent in the manufacture of a composition exhibiting improved chemical and/or physical stability of Leukaemic Inhibitory Factor (LIF) or a derivative or homologue thereof.

Preferred compositions in accordance with the present invention are referred to as "pharmaceutical compositions" where LIF or its derivatives or homologues is/are present in a pharmaceutically acceptable composition.

and includes single or multiple amino acids substitutions, deletions and/or additions to or in the natural, synthetic or recombinant LIF molecule as well as hyperglycosolated and deglycosolated forms. Conditions for preparing recombinant LIF are disclosed in International Patent Application Nos PCT/AU88/00093 and PCT/AU90/00001 although these conditions may vary depending on the host cell used. Any such variations and/or modifications are within the scope of the subject invention. The host cells may be eukaryotic (eg. yeast, mammalian, insect, plant etc) or prokaryotic (eg. *Escherichia coli*, *Bacillus* sp, *Pseudomonas* sp etc) cells.

Accordingly, one aspect of the present invention contemplates a composition comprising leukaemia inhibitory factor (LIF) or a derivative or homologue thereof and a stabilizing agent facilitating chemical and/or physical stability of LIF in the composition, additives for maintaining pH and isotonicity and one or more pharmaceutically acceptable carriers and/or diluents.

Another aspect of the present invention provides a composition with improved chemical and physical stability comprising LIF or a derivative or homologue thereof, a stabilizing agent, additives for maintaining pH and isotonicity and one or more pharmaceutically acceptable carriers or diluents under conditions in which aggregation of LIF is reduced.

Still yet another aspect of the present invention provides a composition with improved chemical and physical stability comprising LIF or a derivative or homologue thereof, a stabilizing agent, additives for maintaining pH and isotonicity and one or more pharmaceutically acceptable carriers or diluents under conditions in which deamidation of LIF is reduced.

Analogues and mimetics include molecules which contain non-naturally occurring amino acids or which do not contain amino acids but nevertheless behave functionally the same as or similar to LIF. Natural product screening is one useful strategy for identifying analogues and mimetics. Analogues of LIF contemplated herein also include modifications

peptide synthesis and the use of cross linkers and other methods which impose conformational constraints on the protein molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carboethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid contemplated herein is shown in Table 1.

N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

All these types of modifications may be important to further stabilise LIF in the composition of the present invention.

The compositions of the present invention achieve their stability through judicious choice of pH conditions within the range of ~~between~~ from about 3.5 to about 6.5 ~~inclusive and~~ optionally the presence of one or more suitable stabilizing agents and/or additives. Preferably, the pH range is between from about 4.0 - 6.0 inclusive, ~~more preferably~~ between from about 4.5 to about 5.5 inclusive. Most preferably, the pH of the composition is about 5.0.

Accordingly, another aspect of the present invention provides a composition comprising Leukaemia Inhibitory Factor (LIF) and one or more pharmaceutically acceptable carriers and/or diluents and wherein the composition has a pH of between about 3.5 and 6.5.

Suitable stabilizing agents are known to those skilled in the art and include ~~isotonicity~~ ... or maintain the conformational stability of LIF and

surfactants. It is understood that one agent may possess more than one stabilizing property and more than one agent may be employed to achieve a stabilizing effect.

Suitable ~~isotonicity~~ agents are those which maintain approximately the same osmotic pressure as that of cellular fluids, and are known to those skilled in the art. These may include, but are not limited to, polyhydric alcohols such as sorbitol, pharmaceutically acceptable salts such as NaCl, buffer species, sugars and pharmaceutically acceptable polymeric compounds. Suitable surfactants may be anionic, cationic, amphoteric or non-ionic. Preferred surfactants include fatty alcohols such as lauryl, cetyl and stearyl alcohols, glyceryl esters such as the mono-, di- and triglycerides, fatty acid esters of fatty alcohols and esters of other alcohols such as propylene glycol, polyethylene glycol, sorbitol, sucrose and cholesterol. Other suitable agents include the polysorbates such as polysorbates 20, 40, 60 and 80 and sorbitan ester, polyoxyethylene derivatives and pharmaceutically acceptable polyoxyethylene-polyoxypropylene copolymers. Suitable agents which maintain or increase the conformational stability of LIF are also known to the person skilled in the art and include sugars and polyhydric alcohols.

Suitable buffers for attaining the desired pH of the composition will be known to those skilled in the art and include phosphate, citrate and acetate buffers. Preferred buffers are citrate and acetate.

Yet another aspect of the present invention contemplates a method of preparing a composition comprising Leukaemia Inhibitory Factor or a derivative or homologue thereof and which exhibits reduced deamidation and/or ~~agglutination~~ aggregation of LIF or a derivative or homologue over time said method comprising admixing LIF or its derivative or homologue with a stabilizing agent.

The compositions of the present invention may be suitable for administration in a variety of forms such as, but not limited to, parenteral (e.g. intravenous, intraperitoneal, intramuscular, intradermal), subcutaneous, nasal, rectal, vaginal, topical, buccal and sublingual.

The carrier and other ingredients of the composition must be pharmaceutically "acceptable" in the ~~sense of being~~ compatible with the other ingredients of the composition

and not injurious to the subject. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Compositions of the present invention suitable for oral administration may be presented as a solution an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

Compositions suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia gum; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Compositions for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter.

Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, ~~bactericides~~ preservatives and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a

to detect changes in chemical and physical degradation.

Freeze/thaw studies revealed high solubility of LIF, i.e. no aggregation, in formulations in the pH range of 4.0 - 6.0 examined, the highest being in the pH range of 4.5 to 5.5, with optimized stability at pH 5.0.

Studies of the various solutions over varying periods of storage time (0 to 8 weeks) and at a range of storage temperatures (-80 to 25°C) revealed optimum stability of the solution was achieved in a preferred pH range of 4.5 to 5.5.

The inventors examined a number of pH levels and ~~stabilizing agents~~additives. Samples at pH 4.0, 4.5, 5.0, 5.5 and 6.0 were prepared in Examples 1 and 2, ~~as described~~ hereinafter, and additional ~~stabilizing agents~~additives, Sorbitol, an isotonicity agent, and Polysorbate 80 (also referred to as Tween-80)~~, as a~~ non-ionic surfactant to reduce non-specific adsorption onto surfaces, including glass, were also included. NaCl was also examined as an isotonicity agent.

LIF is present in the compositions of the invention in effective amounts. Effective amounts include from 0.1 mg/ml to 100 mg/ml. Preferred effective amounts are from 10 mg/ml to 10 mg/ml. Particularly preferred amounts range from 400 mg/ml to 1000 mg/ml.

Suitable amounts of surfactant and isotonic agents may range from 0.001 to 30%. Preferably from 0.01 to 10%, even more preferably from 0.01 to 5.0%.

Particularly preferred compositions are those comprising LIF, sorbitol, polysorbate and a citrate or acetate buffer in the preferred ranges described above.

The present invention further provides for the use of a stabilizing agent in the manufacture of a composition exhibiting improved chemical and/or physical stability of Leukaemia

prepared by combining 0.272 parts stock LIF solution and 0.728 parts buffer to give a final LIF concentration of 1.0 mg/ml, a final buffer concentration of 10 mM, a final sorbitol concentration of 5% w/v and a final Polysorbate 80 concentration of 0.01% w/v.

Table 7 displays pH and osmolality (obtained using a Fiske One-Ten Osmometer) values for 0.4 and 1.0 mg/ml LIF samples prepared using the above buffer systems.

V. Freeze/Thaw Cycling

A. Sample Preparation and Methods

LIF samples were prepared by dilution of stock LIF (3.67 mg/ml in 2 mM phosphate buffer, pH 6.8) with acetate or citrate buffer containing sorbitol and polysorbate 80 to give a final buffer concentration of 10 mM, a theoretical pH of 4.0, 4.5, 5.0, 5.5, or 6.0, a final sorbitol concentration of 5% w/v, a final polysorbate 80 concentration of 0.01% w/v and a final LIF concentration of 1 mg/ml (see Section IV). The final pH of each sample was essentially the same as predicted by theory. Solutions (3 ml) were filtered through 0.22 µm sterile filters (Millex GV) with the first 0.5 ml aliquot from the filter being retained as a

While there was some variability in the individual results (most likely due to the dilution step prior to analysis), there were no trends which would indicate loss of LIF upon freeze/thaw cycling.

Figure 5 illustrates the average concentration (as a percentage of the initial concentration) over 5 freeze/thaw cycles for each of the different pH values.

VI. Long Term Stability at -80°C, -20°C, 8°C and 25°C

A. Preparation of Samples for Storage at -80°C and -20°C

Five LIF formulations were prepared by dilution of stock LIF (3.67 mg/ml in 2 mM phosphate buffer, pH 6.42) with acetate or citrate buffer containing sorbitol and polysorbate 80 to give a final LIF concentration of 0.4 mg/ml or 1.0 mg/ml, a final buffer concentration of 10 mM, a final sorbitol concentration of 5% w/v and a final polysorbate 80 concentration of 0.01% w/v (see Section V). The theoretical pH values were pH 4.0 (acetate buffer), 4.5 (acetate buffer), and 5.0 (citrate buffer). The final pH of each sample was essentially the same as predicted by theory.

Under aseptic conditions in a laminar flow cabinet, the formulations were sterile filtered using 0.22 µm Millex GV (Millipore) filters. The first 1.0 ml of each filtrate was set aside and the vial marked accordingly (previous studies identified that approximately 1 ml was required to saturate the filter binding sites using Millex GV filter units). The remaining volume was filtered into a sterile 50 ml polypropylene tube. Aliquots of each formulation (1.15 ml/vial) were transferred using a multiple dispensing Eppendorf pipette with sterile tips into heat sterilised 2 ml glass vials and capped with sterile teflon lined rubber caps which were then crimped. Vials were labelled and duplicate vials of each formulation were retained for the initial analysis. The remaining vials were stored at either -80°C or -20°C.

B. Preparation of Samples for Storage at 8°C and 25°C

Five LIF formulations were prepared by a dilution of stock LIF (3.67 mg/ml in 2 mM phosphate buffer, pH 6.42) with acetate or citrate buffer containing sorbitol and polysorbate 80 to give a final LIF concentration of 0.4 mg/ml or 1.0 mg/ml, a final buffer

Blank		
0.4 mg/ml	Acetate pH 4.0	x2
	Acetate pH 4.5	x2
	Citrate pH 5.0 x2	
1.0 mg/ml	Acetate pH 4.5	x2
	Citrate pH 5.0 x2	
Blank		
Standards	0.2 mg/ml, 0.4 mg/ml, 0.7 mg/ml and 1.0 mg/ml	

Selected samples were also analysed for particulates using a Malvern Instruments Zetasizer 3000 particle size instrument. Samples were withdrawn from the storage vials using a syringe and placed in the sample cuvette. Samples were counted for 120 sec using a 200 μm pinhole (to obtain the maximum signal), 90° scattering angle, and scattering source at 633 nm using a 10 mW He-Ne ion laser.

D. Results

Data pertaining to solution pH, LIF concentration in mg/ml (determined by comparison to LIF standard solutions), and the area % of the main peak relative to the total peak area for all LIF related peaks in the chromatogram analysed using the three chromatographic methods are shown in Tables 8 through 17. None of the samples showed significant shifts in pH over the storage period.

1. Ion Exchange

~~Figures 6 through 15 illustrate~~ IEC chromatograms for samples stored in each of the different buffer systems at 8 and 25°C~~.~~ showed ~~Two~~ two main products ~~were evident~~ for samples prepared in pH 4.0 and 4.5 buffers (eluting at approximately 9 and 10 min) whereas a single main product (eluting at approximately 10 min) was seen in the pH 5.0 samples. At each pH, there was evidence of several minor degradation products in the ion exchange chromatograms, however, due to inadequate resolution between the different

chromatograms for samples stored at -80 and -20°C are not shown as they were similar to the chromatograms at the higher temperatures with degradation products being present at significantly reduced levels.

The IEC results for samples stored at -80, -20, 8 and 25°C ~~are shown graphically in Figures 16 through 18 with the main LIF peak plotted as a percentage of the total area for all LIF related peaks in the chromatogram as a function of storage time. The data illustrate the dependence of LIF stability on pH and temperature.~~illustrate the dependence of LIF stability on pH and temperature (Tables 8-17). The relative stability under each storage condition was similar for the 0.4 and 1.0 mg/ml formulations. The pH 4.0 samples displayed significant variability between the different time points at 8 and 25°C. Re-analysis of selected samples gave similar results to the original values. There was also evidence of degradation at pH 4.0 and 4.5 following storage at -20°C and -80°C. The stability was greatly improved at pH 5 in comparison to pH 4 and 4.5. At pH 5.0 ~~After~~ after 55 days storage at 8°C, approximately 97% of the total peak area was present as the main LIF peak. Following storage at 25°C for 55 days, this value was reduced to approximately 78%. Samples prepared at pH 5 and stored at -80 or -20°C for up to 84 days showed no significant evidence of degradation.

2. Reversed Phase

Representative RP chromatograms are not included as all displayed essentially the same elution characteristics (see Figure 1). In all cases, the chromatograms showed the presence of only one main peak eluting at approximately 36 min.

The RP results for samples stored at -80, -20, 8 and 25°C, wherein the measured concentration was plotted as a function of storage time, illustrated the absence of significant change in the measured concentration over the storage period for each of the buffer and storage conditions utilised.

3. Size Exclusion

SEC chromatograms for the samples as all displayed essentially the same elution characteristics (see Figure 3). In all cases, the chromatograms showed the presence of one main peak eluting at approximately 26 min and a minor peak eluting at approximately 21 min.

The SEC results for samples stored at -80, -20, 8 and 25°C wherein the measured concentration was plotted as a function of storage time, illustrated the absence of significant change in the measured concentration over the storage period for each of the buffer and storage conditions utilised. Using the SEC method, there was no evidence of chain cleavage or crosslinking under the storage conditions studied.

4. Particle Size Analysis

Samples stored for 56 days at -80 and -20°C and for 41 days at 8 and 25°C were analysed for particulates using a laser light scattering instrument. All of the samples analysed displayed a count rate of "0 kCps" which effectively means that the samples contained no particulates (i.e. no signal was measurable).

VII. Summary

These studies demonstrated no notable loss of LIF following freeze thaw cycling of 1.0 mg/ml LIF solution formulations prepared in acetate or citrate buffers (pH 4 to 6) containing 5% w/v sorbitol and 0.01% w/v polysorbate 80. There was no significant loss of LIF on 0.2 μm Sartorius Minisart filters when formulations were prepared at either 0.4 or 1.0 mg/ml in pH 5.0 or 5.5 citrate buffers containing 5% w/v sorbitol and 0.01% w/v polysorbate 80. For the pH 5.0 and 5.5 formulations, there was also no evidence of loss of LIF on the proposed vials, stoppers, or syringes.

At -80°C, there was no significant change in LIF concentrations measured by RP, IEC and SEC methods following storage for 84 days in the pH range of 4 to 5. At -20°C over the storage period, there was no evidence of degradation for formulations prepared at pH 4 and

analysed by IEC, but the remaining formulations were stable under these storage conditions. Generally, 0.4 and 1.0 mg/ml LIF formulations displayed similar stability characteristics under each of the conditions investigated. Formulations prepared at pH 5 were found to be stable for up to 8 weeks when stored at 8°C with minimal loss of the parent compound (~1%) shown by IEC and no loss shown by RP or SEC.

measured osmolality of the final 0.4 mg/ml LIF formulation was 317 mOsm/kg.

B. Citrate Buffer for 1.0 mg/ml LIF Formulations

Solution A: 13.75 mM sodium citrate (Merck #1.06448)
6.88% w/v sorbitol (Sigma Chemicals #S1876)
0.0138% w/v Polysorbate 80 (Sigma Chemicals #P1754)

Solution B: 13.75 mM citric acid (Merck #1.00244)
6.88% w/v sorbitol (Sigma Chemicals S1876)
0.0138% w/v Polysorbate 80 (Sigma Chemicals P1754)

Solutions A and B were mixed to give a final pH of 5.5. Formulations were prepared by combining 0.272 parts stock LIF solution and 0.728 parts buffer to give a final LIF concentration of 1.0 mg/ml, a final buffer concentration of 10 mM, a final sorbitol concentration of 5% w/v and a final Polysorbate 80 concentration of 0.01% w/v. The measured osmolality of the final 1.0 mg/ml LIF formulation was 322 mOsm/kg.

II. Long Term Stability at 8°C and 25°C

A. Preparation of Samples for Storage at 8°C and 25°C

LIF formulations were prepared by dilution of stock LIF (3.67 mg/ml in 2 mM phosphate buffer, pH 6.42) with citrate buffer containing sorbitol and polysorbate 80 to give a final LIF concentration of 0.4 mg/ml or 1.0 mg/ml, a final buffer concentration of 10 mM, a final sorbitol concentration of 5% w/v and a final polysorbate 80 concentration of 0.01% w/v (see Section II). The theoretical pH was 5.5 and the actual pH of each sample was measured and recorded.

Under aseptic conditions in a laminar flow cabinet, the formulations were sterile filtered using 0.22 µm Millex GV (Millipore) filters. The first 1.15 ml of each filtrate was set aside and the vial marked accordingly. The remaining volume was filtered into a sterile 50

vials and capped with sterile teflon lined rubber caps which were then crimped. Vials were labelled and duplicate vials of each formulation were retained for the initial analysis. The remaining vials were stored at either 8°C or 25°C.

B. Sample Analysis

All LIF samples were analysed undiluted along with standards according to the methods described in Example 1. At each time point, 2 vials were withdrawn from the incubators and approximately 200 μl was removed from each using a sterile 1 ml syringe and a sterile needle. These aliquots were placed into polypropylene autosampler vials and sealed with caps containing self-sealing septa to allow repeat injections from the same vial without evaporation. The original glass sample vials were then marked with the time point and placed at -80°C for repeat analysis (if required) or use in other studies.

Autosampler vials were transferred to the autosampler where they were maintained at 4□C throughout the three analytical runs. The same sample and standard autosampler

IV. Results

Data pertaining to solution pH, LIF concentration in mg/ml (determined by comparison to LIF standard solutions), and the area % for the main peak relative to the total peak area for all LIF related peaks in the chromatogram analysed using the three chromatographic methods are shown in Tables 18 and 19. For each set of samples, there was a slight decrease in solution pH of approximately 0.1 unit over the 92 day storage period.

1. Ion Exchange

A single main product (eluting at approximately 9 min) was seen in all samples stored at 8 and 25°. There was evidence of several minor degradation products in the ion exchange chromatograms, however, due to inadequate resolution between the different products, the exact number of products could not be determined. Samples prepared at pH 5.0 (initial study) and those at pH 5.5 (this study) stored at 8°C and 25°C for 8 weeks were compared. The chromatograms were normalised with respect to the retention time for the main peak to take into account slight changes in the chromatography between the two studies. In each case, the product distribution was similar with a higher proportion of the main degradation product noted in the pH 5.5 samples relative to the pH 5.0 samples.

The IEC results for the samples, wherein the main LIF peak was plotted as a percentage of the total area for all LIF related peaks in the chromatogram as a function of storage time illustrated the dependence of LIF stability on temperature. The relative stability under each storage condition was similar for the 0.4 and 1.0 mg/ml formulations. After 92 days storage at 8°C, 95-96% of the total peak area was present as the main LIF peak. Following storage at 25°C for 92 days, this value was reduced to approximately 56-58%.

The IEC stability data (main peak area expressed as a percentage of the total) obtained for samples at pH 5.5 ~~with~~ and that from the previous study with samples prepared at pH 5.0 were compared. At 25°C, a slight increase in the rate of degradation was evident at pH 5.5.

Example 3

I. Sample Preparation

8°C and 25°C LIF Samples

LIF formulations were prepared by a dilution of stock LIF (3.67 mg/ml in 2 mM phosphate buffer) with citrate buffer containing sorbitol or NaCl to give a final LIF concentration of ~~0.05 or~~ 0.4 mg/ml, a final buffer concentration of 10 mM, a final sorbitol concentration of 5% w/v or a final NaCl concentration of 0.9% w/v. The theoretical pH was 5.0 in all cases. Formulations were prepared and filled into vials as described previously.

II. Analytical Methods

Samples and standards were prepared as previously described. Analyses were conducted by RP and SEC and IEC was conducted using the Polycat A column.

SEC

SEC data for 0.05 and 0.4 mg/ml formulations are plotted with the main peak expressed as a % of the total area. There was some variability in the 0.05 mg/ml samples most likely due to the low concentration. There were no real trends for either buffer at 8°C or 25°C.

Freeze-Thaw Cycling

Freeze-thaw cycling studies for pH 5 citrate buffers containing sorbitol or NaCl were analysed by SEC. After the 5th cycle there was a trend toward a decrease in the main peak as a % of the total area and a slight increase in the pre-eluting high molecular weight peak.

Table 20. Stability of 0.4 mg/mL LIF formulations following storage at 8°C measured by IEC.

Storage Time (Weeks)	Citrate/Sorbitol/Tween pH 5.0 (% of initial conc remaining)	Citrate/Sorbitol/Tween pH 5.5 (% of initial conc remaining)	Citrate/Sorbitol pH 5.0 (% of initial conc remaining)	Citrate/NaCl pH 5.0 (% of initial conc remaining)
0	100	100	100	100
2	99.7	99.4	99.9	101.6
4	99.8	99.7	99.7	101.1
6	99.2	99.2	98.2	98.8
8	98.8	98.5	97.0	98.2

Table 21. Stability of 0.4 mg/Ml LIF formulations following storage at 25°C measured by IEC.

Storage Time (Weeks)	Citrate/Sorbitol/Tween pH 5.0 (% of initial conc remaining)	Citrate/Sorbitol/Tween pH 5.5 (% of initial conc remaining)	Citrate/Sorbitol pH 5.0 (% of initial conc remaining)	Citrate/NaCl pH 5.0 (% of initial conc remaining)
0	100	100	100	100
2	96.4	94.1	84.0	91.8
4	93.0	88.0	80.6	86.2
6	88.6	83.0	72.2	81.5
8	78.9	72.9	68.7	76.0

CLAIMS

1. A composition comprising leukaemia inhibitory factor (LIF) or a derivative or homologue thereof and a stabilizing agent facilitating chemical and/or physical stability of LIF in the composition, additives for maintaining pH and isotonicity and one or more pharmaceutically acceptable carriers and/or diluents whrein the pH of the composition is from between 3.5 and 6.5.

2. A composition according to claim 1 wherein the pH of the composition is from between about 4.5 and 6.5.

3. A composition according to claim 2 wherein the pH of the composition is from between 4.5 and 6.0.

4. A composition according to any one of claims 1 to 3 wherein the stabilizing agent facilitates reduced aggregation of LIF.

5. A composition according to any one of claims 1 to 3 wherein the stabilizing agent facilitates a reduction in the deamidation of LIF.

6. A composition according to any one of claims 1 to 5 wherein the stabilizing agent is an agent which increases or maintains the conformational stability of LIF or its derivatives or homologues or functional equivalents thereof.

6A. A composition according to claim 6 wherein the stabilizing agent is a surfactant.

7. A composition according to claim 6 wherein the stabilizing agent is selected from a polyhydric alcohol, a pharmaceutically acceptable salt, a buffer species, a sugar and a pharmaceutically acceptable polymeric compound.

8. A composition according to claim 7 wherein the polyhydric alcohol is sorbitol.

9. A composition according to claim 6 wherein the surfactant is an anionic, cationic, amphoteric or non-ionic surfactant.

10. A composition according to claim 9 wherein the surfactant is selected from a fatty alcohol, a glyceryl ester and a fatty acid ester of a fatty alcohol or other alcohol.

11. A composition according to any one of claims 6 to 10 wherein the stabilizing agent is selected from a polysorbate, a polyoxyethylene derivative and a pharmaceutically acceptable polyoxyethylene-polyoxypropylene copolymer.

12. A composition according to claim 9 wherein the stabilizing agent is polysorbate 20 and/or polysorbate 80.

13. A composition according to claim 7 wherein the buffer species is selected from a phosphate, citrate and acetate buffer.

14. A composition according to claim 13 wherein the buffer species is a citrate or acetate buffer.

15. A composition comprising leukaemia inhibitory factor (LIF), additives for maintaining pH and isotonicity and one or more pharmaceutically acceptable carriers and/or diluents and wherein the composition has a pH of between 3.5 and 6.5.

16. A composition according to claim 15 wherein the composition has a pH of between 4.5 and 6.5.

17. A composition according to claim 16 wherein the composition has a pH of between 4.5 and 6.0.

18. A composition according to claim 15 wherein the aggregation of LIF over time is reduced.

19. A composition according to claim 15 or 16 or 17 wherein the deamidation of LIF over time is reduced.

20. A composition according to claim 15 or 16 or 17 where the pH is maintained by the presence of a buffer species selection from a phosphate, citrate and acetate buffer.

21. A composition according to claim 20 wherein the buffer species is a citrate or acetate buffer.

22. A composition according to claim 1 or 15 wherein LIF is present in an amount from about 0.1 μg/ml to about 100 mg/ml.

23. A method for preparing a composition comprising leukaemia inhibitory factor (LIF) or a derivative or homologue thereof and which exhibits reduced deamidation and/or aggregation of LIF or its derivative or homologues over time said method comprising admixing LIF or its derivative or homologue with a stabilizing agent and additives for maintaining pH and isotonicity.

24. A method according to claim 21 wherein the stabilizing agent is an agent which increases or maintains the conformational stability of LIF or its derivatives or homologues or a surfactant or functional equivalents thereof.

25. A method according to claim 24 wherein the stabilizing agent is selected form a polyhydric alcohol, a pharmaceutically acceptable salt, a buffer species, a sugar and a pharmaceutically acceptable polymeric compound.

26. A method according to claim 25 wherein the stabilizing agent is selected from a polysorbate, a polyoxyethylene derivative and a pharmaceutically acceptable polyoxyethylene-polyoxypropylene copolymer.

27. A method according to claim 26 wherein the polysorbate is polysorbate 20 and/or polysorbate 80.

28. A method according to claim 25 wherein the polyhydric alcohol is sorbitol.

29. A method according to claim 24 wherein the surfactant is an anionic, cationic, amphoteric or non-ionic surfactant.

30. A method according to claim 28 wherein the surfactant is selected from a fatty alcohol, glyceryl ester and a fatty acid ester of a fatty alcohol or other alcohol.

31. A method according to claim 25 wherein the additives for maintaining pH and isotonicity are selected from a phosphate, citrate and acetate buffer.

32. A method according to claim 31 wherein the additives for maintaining pH and isotonicity are citrate or acetate buffer.

33. A method according to any of claims to 23 further comprising adjusting the pH to between from about 3.5 and about 6.5.

34. A method according to claim 33 further comprising adjusting the pH to between from about 4.5 and about 6.5.

35. A method according to claim 33 further comprising adjusting the pH to between from about 4.5 and 6.0.

36. A method according to to 23 further comprising admixing one or more pharmaceutically acceptable carriers and/or diluents.

37. A method of preparing a composition comprising leukemia inhibitory factor (LIF) or a derivative or homologue thereof which exhibits improved chemical or physical stability of LIF, said method comprising admixing LIF or its derivative or homologue with a stabilizing agent.

38. A method according to claim 37 wherein the stabilizing agent isselected from a polyhydric alcohol, a pharmaceutically acceptable salt, a buffer species, a sugar and a pharmaceutically acceptable polymeric compound.

39. A method according to claim 38 wherein the polyhydric alcohol is sorbitol.

40. A method according to claim 38 wherein the surfactant is an anionic, cationic, amphoteric or non-ionic surfactant.

41. A method according to claim 40 wherein the surfactant is selected from a fatty alcohol, glyceryl ester and a fatty acid ester of a fatty alcohol or other alcohol.

42. A method according to claim 37 where the stabilizing agent is selected from a polysorbate, a polyoxyethylene derivative or a pharmaceutically acceptable polyoxyethylene-polyoxypropylene copolymer.

43. A method according to claim 38 wherein the buffer species is selected from a phosphate, citrate and acetate buffer.

44. A method according to claim 43 wherein the buffer species is a citrate or acetate buffer.

45. A method according to any one of claims 37 to 44 where the pH of the composition is between from about 3.5 to about 6.5.

46. A method according to claims 45 wherein the pH is between from about 4.5 and about 5.5.

47. A method according to claim 46 wherein the pH is between from about 4.5 and about 6.0.

48. A composition according to claim 14 wherein LIF is present in an amount from about 0.1 μg/ml to about 100 mg/ml.